cells expressing GFP from a transfected transgene do not disclose the expression of a therapeutic protein such that it may be isolated and purified; (4) lack of enablement is not merely a matter of isolating transfected PGCs and EGs, but rather the lack of a disclosure specific to transfecting such cells and the lack of information relating to construct design sufficient for expression that is high enough for purification; and (5) Applicant has failed to supply a sufficient scientific basis for the assertion that Chang et al cited by the Examiner cannot be read as requiring limiting the scope of the invention to exclude species other than chicken merely because Chang et al does not disclose the combination of growth factors without a feeder layer. Applicants respectfully traverse each basis for the maintained rejection.

First, the Examiner again asserts that the specification does not state in which manner transfected PGCs and EGs would be made and it is not otherwise apparent. Applicants respectfully maintain that the specification need not teach, and preferably omits, what is well known in the art. This tenet has been reiterated by the Federal Circuit on numerous occasions, inlcuding the following cases to name a few: *In re Buchner*, 929 F.2d 660, 661, 18 USPQ2d 1331, 1332 (Fed. Cir. 1991); *Hybritech, Inc. v. Monoclonal Antibodies* , Inc ., 802 F.2d 1367, 1384, 231 USPQ 81, 94 (Fed. Cir. 1986), cert. denied, 480 U.S. 947 (1987); *and Lindemann Maschinenfabrik GMBH v. American Hoist & Derrick Co.*, 730 F.2d 1452, 1463, 221 USPQ 481, 489 (Fed. Cir. 1984).

Applicants presented Vick and Simkiss (1993) as evidence that it has been known in the art for at least seven years that avian PGCs can be transfected, for example, with retroviral vectors. While Applicants acknowledge the Examiner's argument that Vick and Simkiss do not disclose high level production of a specific *therapeutic* protein in the transgenic chimeric avians they reported, it is applicants' understanding that the use of a marker gene, such as the neo or lacZ genes used by Vick and Simkiss, is a common means of testing – and showing – that transfection and expression of a transfected DNA construct is feasible. Certainly transfection and expression of any protein of interest, including therapeutic proteins, could be accomplished without undue experimentation given the transfection and expression of marker genes demonstrated by those of skill in the art. Applicants respectfully fail to see the scientific basis for asserting that expression of a

therapeutic protein or any protein of interest would be so different than the marker constructs of Vick and Simkiss as to rise to the level of undue experimentation.

Furthermore, the Examiner's argument seems unusually stringent given Applicants' demonstration of GFP expression in transfected PGCs according to the invention. Indeed, GFP is a convenient marker commonly used for monitoring gene expression in transfected cells. The Examiner neglects to consider that "marker" genes also encode proteins, and it is therefore unclear why expression of a marker gene such as GFP is not a sufficient way to demonstrate that protein expression from a transfected DNA construct is feasible in the cells of the invention.

In fact, the levels of a marker gene can be monitored just like the levels of any protein expressed by a transfected DNA construct according to methods known in the art. For instance, according to the attached abstract by Bierhuizen et al., the levels of GFP expression may be measured according to the relative intensities of peak green fluorescence. Accordingly, it is well within the arsenal of those skilled in the art to compare the level of GFP expression achieved by transfecting various vectors into the PGCs of the invention, and choose the one that achieves the most desirable levels for any therapeutic protein of interest. Such an assay is particularly feasible given that Applicants have developed a system for maintaining PGCs long term in culture.

In this regard, Applicants reiterate that the goal of the invention was to develop a long term culture system for avian PGCs that would *facilitate* the production of transgenic and chimeric avians. The production of such transgenic and chimeric avians had already been accomplished and reported in the prior art; the present invention merely makes it easier to accomplish what was already possible. Applicants need not include disclosure specific to transfecting such cells because such transfection procedures were known. Likewise, Applicants need not include information relating to construct design sufficient for expression for expression of proteins, because such constructs were known. Again, according to the Federal Circuit, Applicants need not include and preferably omit that which is known in the art.

Finally, the Examiner asserts that Applicants have failed to provide a sufficient basis for challenging the Examiner's reliance on Chang et al. In the previous Office Action dated

December 22, 1999, the Examiner had argued that the invention could not be applied to species other than chicken PGCs because Chang et al reported no affect of human SCF on chicken cells. Accordingly, it was the Examiner's opinion that the scope of the invention must be limited to the chicken PGCs disclosed in the specification (and not to turkey or the Gallinacea genus) because Chang et al was allegedly evidence of unpredictablity. In the Reply filed May 22, 2000, Applicants noted that, despite the results reported by Chang et al, Applicants in fact use human SCF in the culturing method of the invention, which is in direct contrast to the results reported in Chang et al. In addition, Applicants provided a reasonable explanation as to why the results in Chang et al differed from Applicants' results, i.e., that Chang et al employed a feeder layer and feeder layers sometimes produce inhibitory molecules that have a detrimental affect on cell culture.

Applicants can think of no stronger scientific basis for disputing the applicability of Chang et al as evidence of unpredictability than by pointing out that Applicants own results are contrary to those reported in the reference. If the Examiner has some reason for doubting Applicants' invention, then the case law indicates that it is the Examiner who must have a strong scientific basis for such an attack. See *In re Marzocchi*, 439 F.2d 220, 224, 169 USPQ 367 , 370 (CCPA 1971). Reliance on a reference, such as the Chang reference, that clearly does not report the same culture conditions as those of the invention and moreover utilizes a feeder layer of cells which arguably introduces a wide variety of unknown factors into the medium, does not provide a sufficient scientific basis for doubting Applicants' invention in the first place. If the Examiner prefers that Applicants' rebuttal be in the form of a declaration, Applicants would be more than willing to supply such evidence. Otherwise, the fact that Applicants use human SCF in the claimed method for culturing avian PGCs certainly justifies the scope of the claims, because there is no reason to doubt that human SCF would affect turkey PGCs or PGCs from any Gallinacea species any differently than it does chicken PGCs in the context of the present invention.

In view of all the remarks made above, reconsideration and withdrawal of the rejection under 35 U.S.C. §112, first paragraph is respectfully requested.

Next, claims 1-4, 6-12, 14, 15, 17, 20, and 23 were rejected under 35 U.S.C. §103(a) as being unpatentable over Pain et al in view of Laboskey et al, Han et al and Godin et al.

Essentially, it is the Examiner's opinion that it would have been obvious to affect long term culture of avian PGCs on the claimed combination of growth factors in view of Pain's use of the four growth factors, despite the fact that Pain used a feeder layer in addition to other ingredients (anti-IL11 and anti-retinoic acid antibody) to culture an unpure mixture of blastoderm cells. The Examiner believes it would have been obvious in view of Laboskey's use of three of the four growth factors in a method of culturing murine PGCs, and in view of Han's disclosure that various artisans had reported methods of culturing and transfecting PGCs with retroviral vectors (despite the fact that Han does not disclose specific culturing conditions), and further in view of Godin et al which discloses that the culturing of murine PGCs with SCF in the absence of a feeder layer causes a "large increase in the initial survival and apparent mobility of PGCs in culture." Applicants respectfully traverse this rejection.

First, Applicants respectfully reiterate that the skilled artisan would not be motivated to combine the cited references because they deal with different species of PGCs. Indeed, Labosky et al and Godin et al concern the culture of murine PGCs, not avian PGCs, and there is no reason to expect that a culturing method for murine PGCs would work for avian PGCs. In fact, notwithstanding the fact that the Examiner questions the species specificity of different mammalian growth factors in her enablement rejection, i.e., by citing Chang et al, despite Applicants' use of human SCF for culturing avian PGCs, she nevertheless combines references that deal with culturing very different species of PGCs (avian versus mammalian) as allegedly rendering Applicants' methods obvious. Applicants respectfully submit that these rejections are inconsistent and to maintain such an obviousness rejection would be inequitable. Indeed, Chang et al is more applicable to support the non-obviousness of the invention than it is was for the Examiner's position on enablement, because it teaches away from Applicants' invention and discloses that human SCF does not affect chicken PGCs when.in fact Applicants have shown that it does.

Furthermore, Godin et al is also not applicable to facts at issue. The Examiner applies Godin essentially to argue that those of skill in the art would not have believed that a feeder layer was necessary for culturing avian PGCs, in order to discount Applicants' inclusion of this limitation in the amended claims submitted with the previous Reply. However, it would appear that the entire Godin reference may not have been considered (because only an

abstract was attached to the Office Action). Upon review of the entire reference (attached to this Reply for the Examiner's convenience), it can be seen that Godin in fact teaches that the cells were only sustained for 48 hours in the absence of a feeder layer. Moreover, it is stated on page 807 of the reference (right hand column) that "[t]his may be due to a requirement for an additional factor provided by STO cells," i.e., the feeder cells. The skilled artisan certainly would not read this reference as teaching that feeder cells were dispensable for long term culture of PGCs.

In fact, if the art as a whole is considered (as it must be for an analysis under 35 U.S.C. §103(a)), it is clear that those of skill in the art at the time of the invention believed that feeder layer cells were essential for long term maintainence of PGCs in culture. For instance, Karagene and Petitte (abstract attached to this Reply) noted in a year 2000 reference that "Previous observations obtained from a culture of blastodermal cells on a mouse fibroblast feeder layer (STO) suggested that STO cells provide a factor or factors that facilitate development of avian primordial germ cells (PGC) from dispersed embryo cells. The purpose of the current study was to test the hypothesis that soluble factors produced by STO cells are responsible . . .". If it was still the hypothesis in 1999-2000 that STO feeder cells produce something required for avian PGCs (which is well after the filing date of this application), then it is not clear to applicants how the Examiner can argue that culturing avian PGCs in the absence of a feeder layer was obvious at the time the present invention was made. Moreover, Godin et al does not contradict the non-obviousness of the absence of a feeder layer for long term culture of PGCs because Godin's PGCs lasted only two days in culture without a feeder layer.

In view of all the remarks submitted above, reconsideration and withdrawal of the rejection under 35 U.S.C. §103(a) is respectfully requested.

This Reply is fully responsive to the Office Action dated August 1, 2000. Therefore, a Notice of Allowance is next in order. If the Examiner would like to discuss any aspect of

this Reply or any further issues relating to the subject application, she is encouraged to contact the undersigned so that allowance of the application may be expedited.

Respectfully submitted,

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Date: January 2, 2001

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ACKNOWLEDGEMENTS. We thank members of our laboratory for discussion. W.M.E. was supported in part by a postdoctoral immunology fellowship, B.C. was supported by a postdoctoral fellowship from the Swiss National Science Foundation, and R.J.B. is a Howard Hughes Medical Institute Physician Research Fellow.

TABLE 1 Overall numbers of 8.5- and 10.5-d.p.c. PGC cultured on STO cells are increased by SCF

	Day 1	Day 5
8.5-d.p.c. PGC in SF-1	32.7 (2.7)	322.0 (40.4)
8.5-d.p.c. PGC in SCF	38.6 (4.8)	537.0 (83.7)
P value from unpaired *t*-test	0.1-0.375	0.025-0.05
	Day 1	**Day 3**
10.5-d.p.c. PGC in SF-1	157.6 (24.9)	171.5 (9.6)
10.5-d.p.c. PGC in SCF	179.5 (13.0)	256.7 (3.1)
P value from unpaired *t*-test	0.1-0.375	0.0005

Mean PGC numbers were compared using unpaired *t*-tests.

Effects of the *steel* gene product on mouse primordial germ cells in culture

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MUTATIONS at the *steel* (*sl*) and *dominant white spotting* (*W*) loci in the mouse affect primordial germ cells (PGC), melanoblasts and haemopoietic stem cells[1]. The *W* gene encodes a cell-surface receptor of the tyrosine kinase family[2,3], the proto-oncogene c-*kit*. *In situ* analysis has shown c-*kit* messenger RNA expression in PGC in the early genital ridges[4]. The *Sl* gene encodes the ligand for this receptor, a peptide growth factor, called here stem cell factor (SCF)[5-7]. SCF mRNA is expressed in many regions of the early mouse embryo, including the areas of migration of these cell types[8]. It is important now to identify the role of the *Sl–W* interaction in the development of these migratory embryonic stem cell populations. Using an *in vitro* assay system[9], we show that SCF increases both the overall numbers and colony sizes of migratory PGC isolated from wild-type mouse embryos, and cultured on irradiated feeder layers of STO cells (a mouse embryonic fibroblast line). In the absence of feeder cells, SCF causes a large increase in the initial survival and apparent motility of PGC in culture. But labelling with bromodeoxyuridine shows that SCF is not, by itself, a mitogen for PGC. SCF does not exert a chemotropic effect on PGC in *in vitro* assays. These results suggest that SCF *in vivo* is an essential requirement for PGC survival. This demonstrates the control of the early germ-line population by a specific trophic factor.

SCF was first tested at concentrations between 10 and 100 ng ml^{-1} for its effect on overall numbers of PGC taken from early (8.5-days postcoitum (d.p.c.)) or late (10.5-d.p.c.) in their migratory period, and cultured as described previously[9] on feeder layers of STO fibroblasts. In each case the overall numbers of PGC were increased by SCF after 3-5 days in culture (Fig. 1 and Table 1). Maximum effect was seen with doses between 30 and 40 ng ml^{-1} in serum-free medium, and so this concentration range was used thereafter.

After about 3 days in culture on STO cells, PGC form discrete colonies (Fig. 2*a*). We counted both the numbers of these, and their sizes (as measured by the number of PGC) in the presence or absence of SCF. In two separate experiments SCF increased the numbers and sizes of colonies after both 3 and 5 days in culture (Fig. 2*b*). Labelled bromodeoxyuridine (BRDU) analysis (Fig. 2*c*) showed that the division rate of PGC did not alter throughout 4 days of culture, and was not affected by SCF. As SCF does not increase the rate of division, it must act by increasing the numbers of PGC that survive and proliferate. This does not exclude the possibility that SCF can also stimulate proliferation in combination with other growth factors, as is the case with haemopoietic stem cells[6,10,11].

PGC were also cultured in the absence of STO cells, directly on plastic. These cultures contain contaminating somatic cells from the migratory path, which are known to release SCF *in vivo*[8]. Despite this, added soluble SCF had a large effect on initial survival and motility of 10.5-d.p.c. PGC (Fig. 2*d*). But this effect was only sustained for 48 h, and there was no increase in overall PGC numbers as seen on STO cells. This may be due to a requirement for an additional factor provided by STO cells, or for the presentation of SCF in a membrane-bound form by STO cells. The latter possibility is suggested by the results of Dolci *et al.*[12].

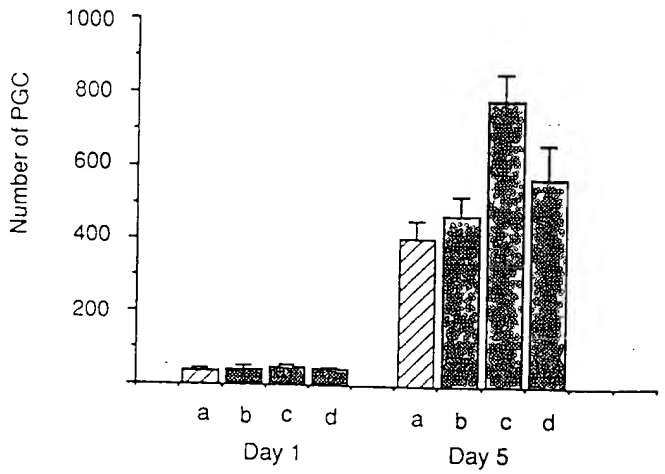

FIG. 1 PGC were removed from 8.5- or 10.5-d.p.c. MF1 embryos, and cultured on irradiated STO feeder cell layers in serum-free medium (SF-1; Northumbria Biologicals). Cultures were fixed after 1, 3 or 5 days and PGC counted by first staining them with alkaline phosphatase. Five replicate samples were counted in each case, and results expressed as means with standard errors. A sample experiment is shown in which 8.5-d.p.c. PGC were cultured in different concentrations of SCF. Peak response was seen with 37.5 ng ml^{-1}, and this concentration was used thereafter. Four duplicate experiments were done with 8.5-d.p.c., and three with 10.5-d.p.c. PGC. Overall means from these experiments, plus standard errors of the means are shown in Table 1. a, SF-1 medium; b, 18.8 ng ml^{-1} SCF; c, 37.5 ng ml^{-1} SCF; d, 75 ngml^{-1} SCF.

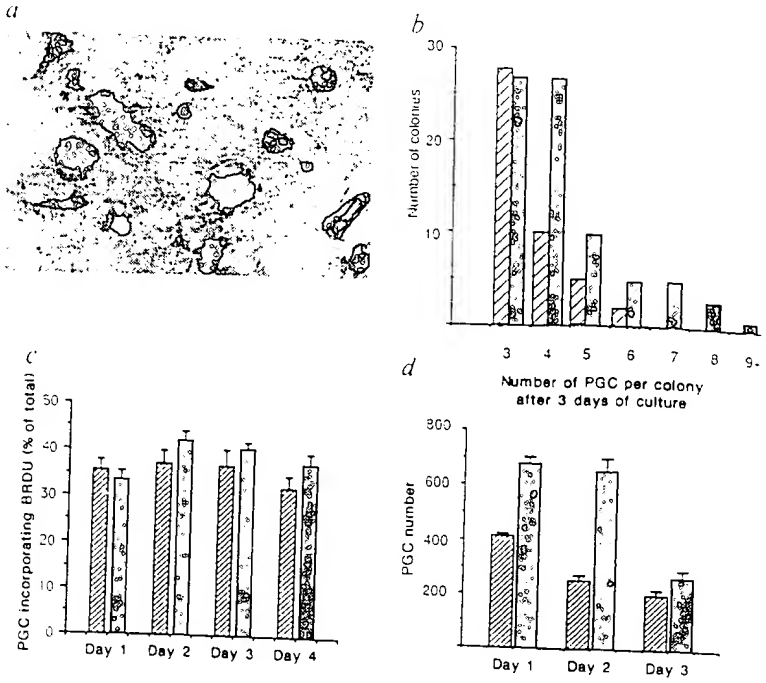

FIG. 2 *a*, Colonies of PGC formed after 3 days' culture on STO cells. PGC in colonies were counted at 3 and 5 days of culture. The colony size distribution for one 3-day experiment is shown in *b*. Total number of colonies in this experiment were 45 in serum-free medium and 79 in 37.5 ng ml^{-1} SCF. At 5 days, total colony numbers were 45 and 90, respectively. This experiment was repeated with near identical results. *c*, Incorporation of BRDU into PGC after a 4-h incubation period, during each day of culture on STO cells. Five assays were done at each time point, and the results expressed as percentage of PGC containing BRDU, together with standard errors. *d*, In the absence of STO cells, SCF stimulates initial survival of PGC. PGC (10.5-d.p.c) were cultured at high concentration (two embryo equivalents per microtitre well) with and without 37.5 ng ml^{-1} SCF. SCF increases the number of adherent PGC, and the number that show a motile phenotype[14]. Five duplicate cultures were counted on each day. Hatched bars, SF-1 medium only; dark bars, SF-1 medium +37.5 ng m^{-1} SCF.

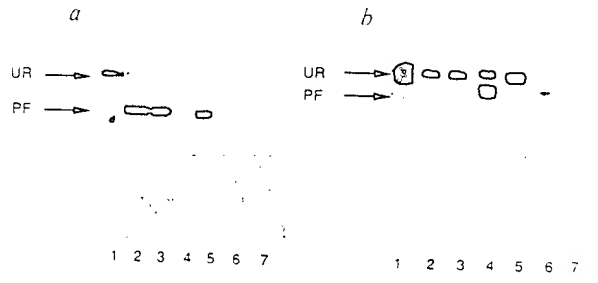

FIG. 3 STO cells express SCF but not c-*kit* mRNA. *a*, SCF protection assay; *b*, c-*kit* assay. UR, undigested ripoprobe; PF, protected fragment. The lanes received the same loadings for each assay: 1, untreated riboprobe transcript; 2, 3T3 cells; 3, fetal liver; 4, fetal cerebellum; 5, STO cells; 6, FDCP-mix, undifferentiated; 7, FDCP-mix, differentiated. STO cells show a protected fragment of the expected size in the SCF assay, but not in the c-*kit* assay. STO cell RNA was prepared by lysis in 4 M guanidine isothiocyanate followed by centrifugation in caesium chloride. RNA probes were prepared from a 396-base pair (GP) fragment of a murine SCF cDNA and a 1,035-bp fragment of a murine SCF c-*kit* cDNA cloned into pGEM vectors following manufacturer's instructions (Promega). Following gel purification, 5–10 × 10^5 c.p.m. of probe was mixed with 20 μg cellular RNA precipitated and resuspended in 20 μl hybridization buffer, and protection assays carried out as in ref. 15, except that RNase digestion buffer was 0.3 M sodium acetate, 10 mM Tris–HCl, pH 7, 5 mM EDTA, 20 μg ml^{-1} RNase A and 50 units ml^{-1} RNase T1.

To eliminate the possibility that the effects seen on STO cells are mediated indirectly by the STO cells, we tested for the presence of c-*kit* mRNA in STO cells. As a positive control for the c-*kit* assay, we used RNA from FDCP-mix (for factor-dependent cells, Patterson-multipotent). These primitive haemopoietic stem cells express c-*kit* in the undifferentiated, but not in the differentiated state[13]. STO cells do not express c-*kit* mRNA, but do express SCF mRNA (Fig. 3). As the c-*kit* protein is the only known receptor for SCF, it is extremely unlikely that the effects of SCF in these assays is being mediated indirectly. The expression of SCF by STO cells suggests that the added SCF in these assays is enhancing a preexisting low-level signal, and would explain our original observation that STO cells promote the survival of PGC *in vitro*[14].

To test whether SCF has a chemotropic effect on PGC, we used an assay[9] in which the genital ridges (the normal target of PGC migration) exert a chemotropic effect on PGC in culture, whereas other tissues tested had no effect. SCF in concentrations of 10–50 ng ml^{-1} did not exert a chemotropic effect in these assays, whereas medium conditioned by 10.5-d.p.c. genital ridges did so (data not shown). Soluble SCF is therefore unlikely to be the factor released by genital ridges in culture. SCF could control migration in other ways. In membrane-bound form its stimulation of motility (shown here) or control of adhesion could provide directional cues for the PGC.

So why do mutations at the *W* and *Sl* loci cause fewer PGC to colonize the genital ridges? These results suggest that when the supply of SCF to the migrating PGC population is reduced (as in *Sl*), or the response is disabled (as in *W*), then PGC fail to survive. Our chemotropic assays make it unlikely that the *W*–*Sl* interaction guides the PGC to their target, at least through a gradient of diffusible, soluble SCF. This conclusion is reinforced by the fact that in alleles of *W* and *Sl* that almost completely eliminate the PGC population, there is still a small number that reach the gonad. In normal development, a proportion of the PGC never reach the genital ridges, but colonize surrounding regions of the embryo, and die. Our results suggest that the mechanism whereby they die might be due to the absence of SCF in these tissues. Whether or not this is due to the ability of SCF to specifically suppress apoptosis in the germ-line cells is not yet known. But the existence of specific survival factors in spatially limiting supply could be a general mechanism for controlling the populations of migrating stem cell populations.

Received 9 May; accepted 5 July 1991.

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ACKNOWLEDGEMENTS. We thank the Wellcome Trust and the Cancer Research Campaign for financial support.

Requirement for mast cell growth factor for primordial germ cell survival in culture

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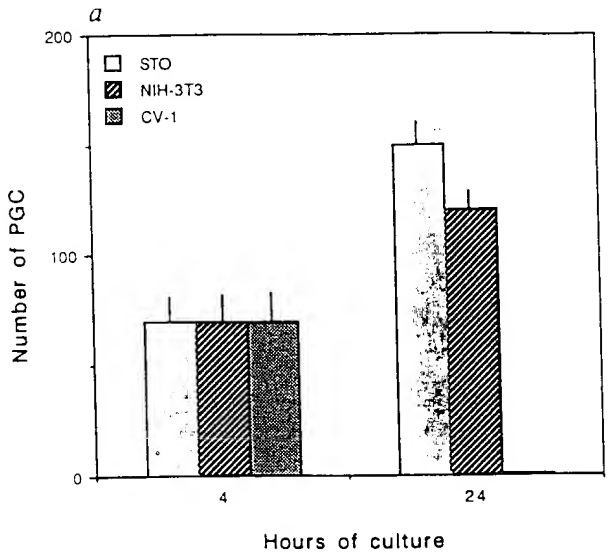
MAST-CELL growth factor (MGF) is encoded by the murine *steel* (*Sl*) locus and is a ligand for the tyrosine kinase receptor protein encoded by the proto-oncogene c-*kit* at the murine *dominant white spotting* (*W*) locus. Mutations at both these loci affect mast cells, primordial germ cells (PGCs), haemopoietic stem cells and melanocytes. In many *Sl* and *W* mutants, the rapid proliferation of PGC that normally occurs between day 7 and 13.5 of embryonic development fails to occur. As c-*kit* is expressed in PGCs[1,2] while MGF is expressed in the surrounding mesenchyme[2,3], MGF might promote the proliferation of PGCs. Here we report that MGF is essential for PGC survival in culture, but does not stimulate PGC proliferation. Moreover, whereas both the transmembrane and soluble proteolytic cleavage forms of MGF stimulate mast-cell proliferation, soluble MGF has a relatively limited ability to support survival of PGCs in culture, thus explaining the sterility in mice carrying the *steel-dickie* (*Sl*^{d}) mutation, which encodes only a soluble form of MGF, and providing a functional role for a transmembrane growth factor.

The ability of MGF to promote survival and/or proliferation of PGCs was determined using a culture system in which both the type and amount of MGF present could be manipulated. Isolated pregonadal (8.5-10.5 days) PGCs survive <24 h in culture in the absence of a confluent monolayer of feeder cells[4]. PGCs do survive when cultured on feeder layers of STO or NIH-3T3 cells[4-6] but not on feeder layers of CV-1 cells (Fig. 1*a*). The differences in PGC survival on STO and NIH-3T3 versus CV-1 feeder layers might be due to differential MGF expression by these feeder cell lines. STO and NIH-3T3 cells both express a 6.5-kilobase (kb) MGF messenger RNA and produce MGF that promotes the proliferation of mast cells, whereas CV-1 cells do neither (Fig. 1*b*; Table 1). CV-1 cells



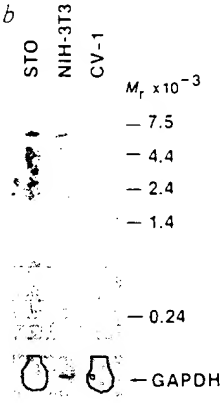


FIG. 1 *a*, Survival of PGCs on STO, NIH-3T3 or CV-1 feeder layers. The number of PGCs present after 4 and 24 h of culture. Bars represent the mean plus/minus the standard deviation of five replicate cultures. Each experiment was done four times. Solid bar, STO; hatched bar, NIH-3T3; dotted bar, CV-1. *b*, Northern analysis of MGF expression by feeder cell lines, STO cells, NIH-3T3 cells and CV-1 cells. RNA loading was assayed by probing for glyceraldehyde 3-phosphate dehydrogenase (GAPDH).
METHODS. STO cells and NIH-3T3 cells are mouse embryo-derived fibroblast cell lines. CV-1 cells are fibroblast-like cells derived from the kidney of a male African green monkey. Cells were grown in DMEM (Gibco) supplemented with 2 mM L-glutamine, 1 mM sodium pyruvate and either 10% FBS (STO and CV-1) or 10% calf serum (NIH-3T3 cells). Staged embryos were derived from timed matings of B6C3F1 animals as described[4], and PGCs isolated from 8.5-day postcoitus (dpc) embryos by dissection of a fragment consisting of the caudal end of the primitive streak and the base of the allantoic rudiment[6]. These fragments were dissociated in 0.05% trypsin, 0.02% EDTA (Sigma) to yield a single-cell suspension of PGC and somatic cells. This cell suspension was plated onto confluent, irradiated, feeder layers as previously described[4]. PGC were maintained in feeder-layer culture in DMEM supplemented with 15% FBS and identified by alkaline phosphatase histochemistry[4,6]. For northern analyses, confluent cell monolayers were washed with PBS, lysed with RNAzol (Cinna/Biotex) and total RNA prepared by the method of Chomczynski and Sacchi[22]. Poly(A)$^+$ RNA was selected using a Fast Track kit (InVitrogen). Poly(A)$^+$ RNA (2 μg) were run on a 1.5% agarose-formaldehyde gel, transferred to Nytran (Schleicher and Schuell) and baked. Northern blots were probed with a 2.0-kb *Sal*I fragment of an MGF cDNA clone (MGF-10), representing the entire MGF coding sequence[15]. The probe was ^{32}P-labelled using a Multiprime labelling kit (Amersham) and hybridizations were done according to Church and Gilbert[23]. To quantitate RNA loading, blots were stripped and reprobed with a GAPDH cDNA probe[24].

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1: *Biochem Biophys Res Commun* 1997 May 19;234 (2):371-5

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Green fluorescent protein variants as markers of retroviral-mediated gene transfer in primary hematopoietic cells and cell

Bierhuizen MF, Westerman Y, Visser TP, Wognum AW, Wagemaker G

Institute of Hematology, Erasmus University Rotterdam, The Netherlands.

Retroviral vectors are widely used for the introduction of exogenous genetic material into hematopoietic cells. Here we report the generation of retrovira vectors containing the Aequorea victoria green fluorescent protein (GFP) ge improved versions thereof. Murine fibroblasts transduced with the mutant G genes demonstrated a distinct green fluorescent signal in fluorescence-activ cell sorter (FACS) analysis. The relative intensities of peak green fluorescen observed with different GFP mutants were in the order EGFP>hGFP(S65T) GFP-PTS1 or RSGFP>wildtype GFP (wtGFP). Furthermore, GFP-PTS1 expression was observed in murine (3T3, Rat2, and freshly-cultured bone m and human (K562) cells transduced with the corresponding retroviral vector GFP-PTS1 positive phenotype could be selected for by FACS and appeared stable for at least 1 month in murine fibroblasts and human K562 cells. The these GFP variants are convenient selectable markers to monitor retroviral-mediated gene transfer and expression in mammalian hematopoietic cells.

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Soluble factors and the emergence of chick primordial germ cells vitro.

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Previous observations obtained from a culture of blastodermal cells on a mouse fibroblast feeder layer (STO) suggested that STO cells provide a factor or factors that facilitate development of avian primordial germ cells (PGC) from dispersed embryo cells. The purpose of the current study was to test the hypothesis that soluble factors produced by STO cells are responsible, at least in part, in supporting the development of PGC in culture and to examine the effect of stem cell factor (SCF), ciliary neurotrophic factor (CNTF), and basic fibroblast growth factor (bFGF) in the development of PGC in culture. Blastodermal cells on gelatin-coated plastic or on feeder layers of CV-1 cells yielded a small number of PGC. When blastodermal cells were cultured on STO cells, a marked increase PGC was observed. The addition of STO cell-conditioned medium (STO-CM) to blastodermal cells cultured on gelatin-coated plastic and on feeder layers of cells resulted in a significant increase in the number of PGC, indicating that soluble factors produced by STO cells can enhance the development of chicken PGC in culture. Supplementation of blastodermal cells with SCF (100 ng/m CNTF (2 ng/mL) or with CNTF and SCF together resulted in a significant increase in the number of PGC after 48 h of culture on feeder layers of CV-1 However, addition of bFGF (100 ng/mL) did not increase PGC. We conclude from these observations that the culture of blastodermal cells on feeder layers of STO and CV-1 cells can be used as a useful biological system in examining the regulatory factors that govern the ontogeny of the germ cell lineage in the avian embryo.

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